CO_2), 184 (M − HNCO), 140, 112.

Anal. Calcd for $C_9H_{11}NO_7$: C, 44.09; H, 4.52; N, 5.71. Found: C, 43.98; H, 4.40; N, 5.58.

5-β-D-Ribofuranosyl-1,3-oxazine-2,4-dione (Oxazinomycin, 1). A solution of 1.298 g (2.46 mmol) of **14** in 35 ml of 90% CF_3COOH was stirred at room temperature for 3 h. The solvents were removed at ca. 30 °C (0.2 mmHg). The residue was dried azeotropically by evaporation from absolute EtOH and purified by chromatography on 200 g of silica gel. The column was developed with AcOEt–AcMe–MeOH–H_2O, 70:10:5:5, and appropriate fractions were evaporated in vacuo at 30 °C. Crystallization of the residue from MeOH containing a small amount of H_2O afforded 387 mg of oxazinomycin (**1**), mp 153–155 °C. The mother liquors, after evaporation, gave an additional 98 mg of **1** from AcMe, mp 152–154 °C, total yield 80%, mmp with an authentic sample[7] 153–155 °C. Occasionally, upon slow recrystallization from water–methanol, a second polymorph was obtained, which had mp 161–162 °C dec (reported[4–6] 161 °C). Synthetic **1** and natural oxazinomycin had identical R_f values in several TLC systems; e.g., in EtOAc–AcMe–H_2O, 70:10:5:5, the R_f was 0.34: $[\alpha]^{25}D$ +15.29° (*c* 0.9942, H_2O); uv max (H_2O) 230 nm (ε 4700); ir (KBr) 3470, 3420, 1797, 1773, 1678 cm^{-1}; NMR (D_2O) δ 3.77, 3.90 (CH_2, 2 dd, J_{vic} = 5, 3, J_{gem} = 12.5 Hz), 4.06 (H-4′, ddd, *J* = 5,3,5 Hz), 4.19 (H-3′, t, *J* = 5, 5 Hz), 4.34 (H-2′, t, *J* = 5, 5 Hz), 4.72 (H-1′, d, *J* = 5 Hz), 7.88 (vinylic, s); MS *m/e* 227 (M − H_2O), 209, 202, 196.

Anal. Calcd for $C_9H_{11}NO_7$: C, 44.09; H, 4.52; N, 5.71. Found: C, 44.22; H, 4.52; N, 5.70.

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Registry No.—**1**, 32388-21-9; **3**, 56779-60-3; **4**, 56703-40-3; **5**, 60526-02-5; **6**, 60526-03-6; **8**, 60526-04-7; **9**, 60526-05-8; **10**, 60526-06-9; **11**, 60526-07-0; α-**12**, 60526-08-1; β-**12**, 60526-09-2; α-**12** keto anomer, 60526-10-5; β-**12** keto anomer, 60526-28-9; **13**, 60526-11-6; **14**, 60526-12-7; **15**, 60526-13-8; diethyl cyanomethylphosphonate, 2537-48-6; 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribose, 55726-19-7; bis(dimethylamino)-*tert*-butoxymethane, 5815-08-7.

References and Notes

(1) Second in a series on C-nucleoside antibiotics. Part 1: S. De Bernardo and M. Weigele, *J. Org. Chem.*, **41**, 287 (1976).
(2) R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley-Interscience, New York, N.Y., 1970.
(3) K. Gerzon, D. C. DeLong, and J. C. Cline, "IUPAC Symposium on Antibiotics", S. Rakhit, Ed., Ste. Marguerite, Quebec, Canada, March 1971, p 489.
(4) T. Haneishi, T. Okazaki, T. Hata, C. Tamura, M. Nomura, A. Naito, I. Seki, and M. Arai, *J. Antibiot.*, **24**, 797 (1971).
(5) K. Sasaki, Y. Kusakabe, and S. Esumi, *J. Antibiot.*, **25**, 151 (1972).
(6) Y. Kusakabe, J. Nagatsu, M. Shibuya, O. Kawagudi, C. Hirose, and S. Shirato, *J. Antibiot.*, **25**, 44 (1972).
(7) K. Isouo and R. J. Suhadolnik, *Ann. N.Y. Acad. Sci.*, **255**, 390 (1975).
(8) G. E. Gutowski, M. J. Sweeney, D. C. DeLong, R. L. Hamill, K. Gerzon, and R. W. Dyke, *Ann. N.Y. Acad. Sci.*, **255**, 544 (1975).
(9) H. Ohrui and J. J. Fox, *Tetrahedron Lett.*, 1951 (1973).
(10) H. Ohrui, G. H. Jones, J. G. Moffatt, M. L. Maddox, A. T. Christensen, and S. K. Byram, *J. Am. Chem. Soc.*, **97**, 4602 (1975).
(11) H. Bredereck, G. Simchen, S. Rebsdat, W. Kantlehner, P. Horn, R. Wahl, H. Hoffmann, and P. Grieshaber, *Chem. Ber.*, **101**, 41 (1968). The aminal ester used for this formylation proved to be much more reactive than the commercially available DMF acetals; cf. also H. Bredereck, F. Effenberger, and H. Botsch, *ibid.*, **97**, 3397 (1964).
(12) S. Trofimenko, *J. Org. Chem.*, **28**, 2755 (1963).
(13) D. V. Gardner and D. E. McGreer, *Can. J. Chem.*, **48**, 2104 (1970).
(14) W. Leimgruber and M. Weigele, U.S. Patent 3 917 632 (1975).

Carbon-13 Nuclear Magnetic Resonance Studies of Fungal Metabolites, Aflatoxins, and Sterigmatocystins

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^{13}C NMR spectra are reported for 12 of the fungal metabolites which contain the fused bisdihydrofuran ring system and are produced by certain strains of *A. flavus*, *A. parasiticus*, and *A. versicolor*. Included are the aflatoxins B_1, B_2, B_{2a}, B_3 (parasiticol), D_1, G_1, G_2, and G_{2a} and sterigmatocystin, dihydrosterigmatocystin, *o*-methylsterigmatocystin, and *o*-methyldihydrosterigmatocystin. Chemical shifts have been assigned on the basis of known substituent effects, off-resonance decoupling experiments, and comparison among the related compounds.

The aflatoxins and related sterigmatocystins, fungal metabolites produced by certain strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus versicolor*, are of considerable interest because of their widespread occurrence in human and animal foodstuffs and their carcinogenic effects in all laboratory animals with which they have been tested.[1,2] The common structural feature of these compounds is the bisfuran ring system, which in the aflatoxins is fused to a substituted coumarin structure. Previous studies have shown that the above compounds are derived biosynthetically from a polyketide (acetate) precursor[3–7] and that sterigmatocystin is a precursor of aflatoxin B_1.[8] Although the structures of these compounds have been elucidated previously,[1] the recent advances in ^{13}C NMR toward smaller sample sizes and the wealth of information available from ^{13}C NMR[9] makes ^{13}C NMR a valuable tool for the identification of these metabolites and the structure determination of future metabolites.

Two reports[4,5] have appeared recently on the ^{13}C NMR spectrum of aflatoxin B_1 in connection with ^{13}C labeling studies into the biosynthetic origin of aflatoxin B_1. However, the two reports differ in their assignment of the carbon chemical shifts of aflatoxin B_1. In view of the importance of the aflatoxins and related compounds, we wish to report here our studies of the ^{13}C NMR spectra of eight related aflatoxins. A consistent assignment of the ^{13}C chemical shifts of the aflatoxins is made which is in agreement with one of the previous assignments.[4] In addition, ^{13}C NMR data are also reported for sterigmatocystin and three derivatives. The results are consistent with a previous assignment of the ^{13}C NMR spectrum of sterigmatocystin.[7]

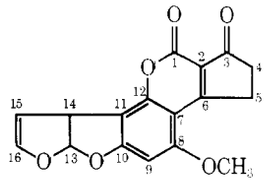
Experimental Section

Natural abundance, proton-decoupled ^{13}C NMR spectra were obtained on a JEOL PFT-100 spectrometer equipped with the JEOL EC-100 data system. Fourier transform spectra were obtained using spectral widths of 5000 and 6250 Hz, with 8K data points. A pulse angle of ~40° was used with a repetition rate of 3 s. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane and are considered accurate to 0.1 ppm. Single frequency, off-resonance proton decoupled (sford) spectra were obtained on each sample.

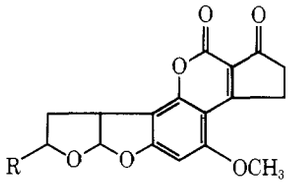
The compounds reported in this investigation were available in one of our laboratories, with the exception of aflatoxin D_1. The sample of aflatoxin D_1 was obtained from the Southern Regional Research Center, New Orleans, La. No impurity peaks were observed in any of the spectra. Samples were prepared in either $CDCl_3$ or Me_2SO-d_6, depending on the solubility. Variations in ^{13}C chemical shifts for $CDCl_3$ vs. Me_2SO-d_6 may be as large as ≥0.5 ppm for all carbons. The concentrations of the NMR samples were from 0.1 to 0.5 M, depending on the quantity of sample available.

Results and Discussion

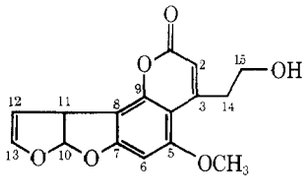
The structures of the compound reported in this investigation are given below. ^{13}C chemical shifts obtained for the



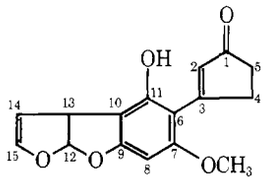
1, aflatoxin B_1



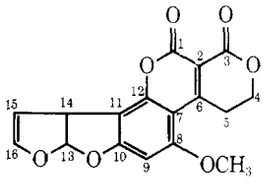
2, R = H, aflatoxin B_2
3, R = OH, aflatoxin B_{2a}



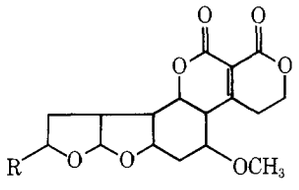
4, aflatoxin B_3



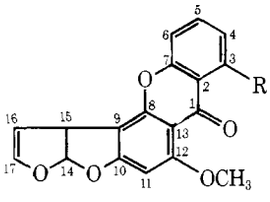
5, aflatoxin D_1



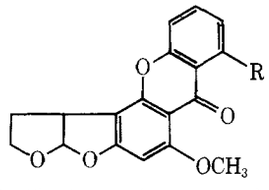
6, aflatoxin G_1



7, R = H, aflatoxin G_2
8, R = OH, aflatoxin G_{2a}



9, R = OH, sterigmatocystin
11, R = OCH_3, *O*-methylsterigmatocystin



10, R = OH, dihydrosterigmatocystin
12, R = OCH_3, *O*-methyldihydrosterigmatocystin

aflatoxins (**1–8**) are given in Table I and those for the sterigmatocystins (**9–12**) in Table II.

The chemical shifts obtained for aflatoxin B_1 (**1**) agree to within ±1 ppm with those reported previously.[4,5] Differences in concentration could account for the minor variations. Comparisons of the spectra of **1, 2,** and **3,** off-resonance decoupling experiments, and the substituent effect of the hydroxy group confirm the previous assignments[4,5] of C-13–16. The previous assignments of the ^{13}C spectrum of **1,** however, differ in their assignments of C-1, C-2, and C-7. The data obtained for **4** and **5** allow a clarification of these assignments.

Comparisons of the spectra of **1** and **4** shows, among other differences, the disappearance of a peak at 117.0 ppm in **1** (sford singlet) and the appearance of a new peak at 110.8 ppm (sford doublet) in **4.** Since the carbon in **1** which should give a doublet in the sford spectrum also shows doublets within experimental error in the spectrum of **4,** the peak at 117.0 ppm in **1** and at 110.8 ppm in **4** can only be due to C-2. This assignment is in agreement with the previous assignment of **1** which was based on carbon–carbon coupling constant data.[4] The disappearance of the peak at 200.6 in **4** compared to **1** and its presence in the spectrum of **5** at 208.6 is consistent with the assignment of this peak to C-3 in **1.** Another major difference in the spectra of **1** and **4** is that the peak at 176.5 ppm (sford singlet) in **1,** which was assigned to C-6,[4] is absent in the spectrum of **4.** It is well established that conjugation of a carbonyl group with a double bond results in a downfield shift of the β carbon of the double bond.[9] Comparison of the spectra of cyclohexene[10] with 2-cyclohexenone[11] shows this downfield shift to be 22.6 ppm. Therefore, the resonance due to C-3 in **4** should be upfield by approximately 22 ppm compared to its position in **1.** Comparison of the spectra of **1** and **4** indeed shows a peak at 154.7 ppm (sford singlet) in **4** which is not present in **1.** Therefore, this peak is assigned to C-3 of **4.**

The remaining discrepancy in the previous assignments of the spectrum of **1** is C-1. The lack of carbon–carbon coupling for the peak at 154.7 ppm in the spectrum of **1** which was grown with $^{13}CH_3{}^{13}CO_2Na$, was used to assign C-13 in one report[4] while the chemical shift of the related carbon in coumarin at 160.4 ppm[12] was used as the basis of the assignment in the other report.[5] The major difference between coumarin and **1** (as far as C-1 in **1** is concerned) is that the α- and β-unsaturated carbons of coumarin are substituted and the carbonyl is not part of a 1,3-dicarbonyl system. Comparison of data for 2-cyclopentenone and 2-cyclohexenone with 2,3-dimethyl-2-cyclopentenone and 2,3-dimethyl-2-cyclohexenone,[11] respectively, indicates that substitution in the α- and β-unsaturated carbons of coumarin should have little effect on the chemical shifts of the carbonyl carbon. However, comparison of data for 2-pentanone with 2,4-pentadione shows that the introduction of a carbonyl group β to an existing carbonyl group results in an upfield shift of ~5 ppm for the carbonyl carbon. Similar results were obtained for ethyl acetoacetate compared to methyl butanoate.[9] Therefore, it seems clear that the resonance of C-1 in **1** should be upfield from the corresponding shift in coumarin (160.4 ppm) and that the assignment (154.7 ppm) based on coupling constant data is the correct assignment.[4] If this analogy is correct, one should observe a downfield shift for C-1 on going from **1** to **4.** Therefore the sford singlet at 158.6 ppm is assigned to C-1 in **4.** The remainder of the carbons in **1, 2, 3,** and **4** were assigned from sford spectra, substituent effects, and from comparison of the spectra.

The carbons in the cyclopentenone ring of **5** (C-1–C-5) were assigned by comparison with the spectrum of 3-methyl-2,3-cyclopentenone.[11] Carbons 9–15 of **5** were assigned by comparison of the spectra of **1–4.** The remaining carbons of **5** were assigned using substituent effects, sford spectra, and comparison with the spectra of **1–4.**

The assignments of the ^{13}C spectra of aflatoxins G_1 (**6**), G_2 (**7**), and G_{2a} (**8**) were based on the assignments of **1, 2,**and **3.** Compared to the spectrum of **1,** the spectrum of **6** should differ principally in the position of the resonances of C-2–C-6 because of the differences between the cyclopentenone and cyclohexenolide rings. The upfield shifts for C-2 and C-6 in **6** compared to **1** are consistent with the difference between an α,β-unsaturated ketone (**1**) and an α,β-unsatrated ester (**6**).[9] Similarly, the differences observed for C-4 and C-5 in

Table I. Carbon-13 Chemical Shifts for Some Aflatoxins[a]

Carbon	1[b]	2[c]	3[c]	4[c]	5[c]	6[b]	7[b]	8[c]
1	154.7	155.8	153.9	[158.6][d]	208.6	154.8	154.9	153.9
2	117.0	115.6	117.4	110.8	131.5	113.2	113.7	111.0
3	200.6	200.0	200.0	154.7	170.9	159.9	160.0	159.6
4	35.0	34.6	34.6	103.5	31.9	64.3	64.3	64.1
5	29.0	28.5	28.5	[158.8][d]	34.2	28.8	28.9	28.4
6	176.5	176.6	176.7	91.0	[106.1][d]	161.1	161.3	161.9
7	103.7	105.5	106.9	160.6	159.5	106.9	102.1	105.3
8	161.0	160.8	160.8	106.8	86.6	161.0	161.0	161.6
9	90.6	90.0	90.5	150.7	158.6	91.0	90.3	91.1
10	165.3	165.8	165.3	112.6	[106.7][d]	164.6	166.1	164.9
11	107.5	105.9	108.6	47.2	151.3	107.5	106.6	108.6
12	152.5	152.0	152.0	102.0	111.5	151.7	152.2	151.6
13	113.2	113.3	113.6	145.1	47.6	113.2	113.7	113.7
14	47.8	42.8	41.3	41.1	103.1	47.7	43.9	41.9
15	102.3	30.6	37.2	59.9	144.1	102.3	31.4	41.9
16	144.8	66.9	99.7			144.8	67.7	91.1
OCH_3	56.4	56.6	56.6	56.4	55.8	56.4	56.4	56.7

[a] In parts per million downfield from Me_4Si. [b] In $CDCl_3$. [c] In Me_2SO-d_6. [d] Assignments may be reversed.

Table II. Carbon-13 Chemical Shifts for Some Sterigmatocystins[a,b]

Carbon	9	10	11	12
1	180.9	180.8	174.6	174.7
2	108.8	108.7	106.2	106.0
3	154.7	154.6	156.5	156.3
4	106.4	105.5	106.2	106.0
5	135.4	135.2	133.4	133.1
6	111.0	110.7	108.9	108.7
7	162.1	161.9	160.4	160.3
8	153.7	154.6	152.9	153.2
9	106.4	106.7	106.2	106.0
10	164.3	165.7	162.7	164.1
11	90.4	89.6	90.3	89.5
12	163.0	163.1	162.7	162.7
13	105.7	105.1	105.6	104.2
14	113.1	113.1	112.9	112.8
15	47.9	44.2	48.1	44.3
16	105.7	31.4	102.6	31.5
17	145.1	67.6	145.0	67.5
CH_3O^-	56.6	56.6	56.3	56.3
CH_3O^-			56.3	56.3

[a] In parts per million downfield from Me_4Si. [b] In $CDCl_3$ solution.

comparing **1** with **6** are consistent with the differences in the data for an ethyl ester (**6**).[9] The upfield shift for C-3 in **6** compared to **1** is in the expected direction. The assignment of the remainder of the carbons in **6**, **7**, and **8** follows from the assignment of similar carbons in **1**, **2**, and **3**.

The assignment of the ^{13}C NMR spectra of sterigmatocystin (**9**, Table II) has been reported previously.[7] Our data for **9** agree with those reported previously, considering that the spectra were obtained on solutions of different concentrations. The assignment of the spectrum of **10** was based on the assignment of **9** and on the differences in the spectra of **1** and **2**. Major differences between the spectra of **9** and **10** were in the chemical shifts of C-15, C-16, and C-17 as expected. The assignment of the spectrum of **11** was based on that of **9** by taking into account the substituent effect difference between the hydroxy and methoxy groups.[9] The upfield shift observed for C-1 in **11** compared to **9** is probably due to a steric difference between the methoxy and hydroxy groups and to the absence of hydrogen bonding to the carbonyl group in **11**.[9] Assignment of the spectrum of **12** follows from that of **11** and the above discussion.

Comparison of the spectra of **1–8** and **9–12**, in conjunction with other data, allows a consistent assignment for the carbons of all compounds to be made. Even though the compounds reported here are similar, there are significant differences in their ^{13}C NMR (Tables I and II) which allow one to distinguish the compounds. These data should prove useful in identifying these fungal metabolites in future investigations.

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Registry No.—**1**, 1162-65-8; **2**, 7220-81-7; **3**, 17878-54-5; **4**, 23315-33-5; **5**, 52373-83-8; **6**, 1165-39-5; **7**, 7241-98-7; **8**, 20421-10-7; **9**, 10048-13-2; **10**, 6795-16-0; **11**, 17878-69-2; **12**, 24945-81-1.

References and Notes

(1) W. B. Turner, "Fungal Metabolites", Academic Press, New York, N.Y., 1971.
(2) L. A. Goldblatt, Ed., "Aflatoxin", Academic Press, New York, N.Y., 1969.
(3) M. Biollaz, G. Buchi, and G. Milne, *J. Am. Chem. Soc.*, **90**, 5017 (1968).
(4) P. S. Steyn, R. Vleggaar, P. L. Wessels, and D. B. Scott, *J. Chem. Soc., Chem. Commun.*, 193 (1975).
(5) D. P. H. Hsieh, J. N. Seiber, C. A. Reece, D. L. Fitzell, S. L. Yang, J. I. Dalezios, G. N. LaMar, D. L. Budd, and E. Motell, *Tetrahedron*, **31**, 661 (1975).
(6) H. Seto, L. W. Cary, and M. Tanabe, *Tetrahedron Lett.*, 4491 (1974).
(7) K. G. R. Pachler, P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Chem. Commun.*, 355 (1975).
(8) D. P. H. Hsieh, M. T. Lin, and R. C. Yao, *Biochem. Biophys. Res. Commun.*, **52**, 992 (1973).
(9) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972.
(10) R. G. Parker and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 743 (1970).
(11) D. H. Marr and J. B. Stothers, *Can. J. Chem.*, **43**, 596 (1965).
(12) L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra", Wiley, New York, N.Y., 1972.